UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

August 07, 2015

[redacted]

MEMORANDUM

SUBJECT: **Human Health Tests evaluation for P-15-0487-491:**

1) Acute oral toxicity study in rats
2) Acute dermal toxicity study in rats
3) Acute inhalation toxicity study in rats
4) 28-day inhalation toxicity study in rats
5) 90-day inhalation toxicity study in rats
6) In vitro genetic mutation study in bacteria
7) In vivo bone marrow erythrocyte micronucleus test in mice
8) In vitro mammalian chromosome aberration

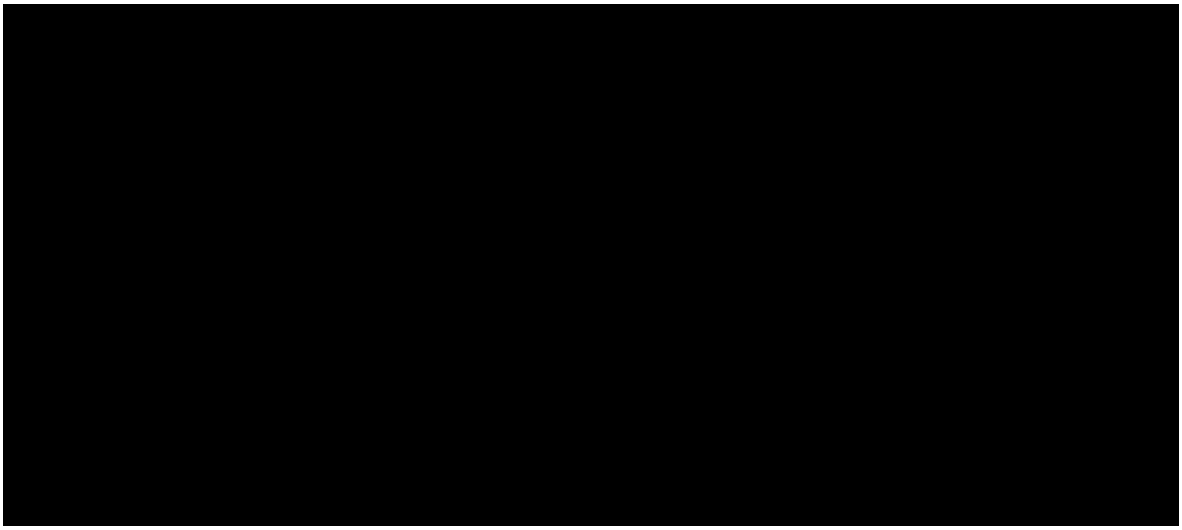
FROM: Viktor Morozov, Ph.D.
Assessment Branch 3
Risk Assessment Division (7403M)
(Reviewed by SRC with RAD Quality Assurance)

TO: James Alwood, Program Manager
New Chemicals Management Branch
Chemical Control Division (7405M)

THRU: Louis Scarano, Ph.D., Branch Chief
Assessment Branch 1
Risk Assessment Division (7403M)

DATA EVALUATION RECORD

Submitter name: Daewoo International USA Corp.
Chemical identity:



P-15-0487-0491

Multi-walled carbon nanotubes; Trade name: K-Nanos-100P Grade, K-Nanos-100T Grade; Purity: >90% for all studies.

Executive Summary:

In an acute oral toxicity study, a group of three female Sprague-Dawley (SD) rats were administered P-15-0487 (purity: >90%) in DPPC solution via gavage at 300 mg/kg-bw and observed for 14 days. No mortality or clinical signs of toxicity were observed within 6 days in the first group of rats, so a second group of three female rats received the same dose and were observed for 14 days. No mortality or clinical signs of toxicity were noted. Body weight gain was normal in all animals throughout the study period. Necropsy revealed no macroscopic abnormalities. The acute oral LD50 was > 300 mg/kg-bw in female rats.

In an acute dermal toxicity study, Sprague-Dawley CD rats (5/sex/group) were dermally exposed to a single dose of P-15-0487 (purity: > 90%) at 0 or 2000 mg/kg-bw and observed for 14 days. P-15-0487, moistened with DPPC solution, was applied to the clipped, intact skin of each rat and held in place for 24 hours under semi-occlusive dressing. Following the exposure period, the dressings were removed and the test site was washed with sterile distilled water. No mortalities occurred and no signs of toxicity or skin irritation were noted during the study. No treatment-related effects on body weight were observed. Necropsy revealed no macroscopic abnormalities. The acute dermal LD50 was > 2000 mg/kg-bw in male and female rats.

In an acute inhalation toxicity study, Fisher 344 rats (5/sex/group) were exposed whole-body to P-15-0487 (purity: > 90%) for 6 hours at measured concentrations of 0 (filtered

fresh air), 0.00017, 0.00052, and 0.00083 mg/L and were observed for 14 days post-exposure. No treatment-related mortality or signs of toxicity were observed during the study. There were no significant effects on body weight. No treatment-related effects were observed at necropsy. The 6-hour LC50 was > 0.00083 mg/L in male and female rats.

In a 28-day repeated-dose inhalation toxicity study, Fischer 344 rats (10/sex/group) were exposed, nose-only, to P-15-0487 (purity: > 90%) at measured concentrations of 0, 0.00017, 0.00051, and 0.00097 mg/L for 6 hours/day, 5 days/week, for 28 days. No mortalities or PMN substance-related adverse effects were observed. The NOAEC for male and female rats was > 0.00097 mg/L.

In a 90-day repeated-dose toxicity inhalation study, Fischer 344 rats (10/sex/group) were exposed, nose-only, to P-15-0487 (purity: > 90%) at measured concentrations of 0 (filtered fresh air), 0.00017, 0.00051, and 0.00101 mg/L for 6 hours/day, 5 days/week, for 13 weeks. Additional male rats (5/group) were included in the control, low-, mid-, and high-concentration groups for the assessment of recovery after a 13-week non-exposure period. No PMN substance-related mortalities or adverse effects were observed. The NOAEC for male and female rats was > 0.00101 mg/L.

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed to P-15-0487 (purity: > 90%) in [redacted] solution at concentrations ranging from 31 to 500 µg/plate, with and without metabolic activation. Vehicle and positive controls were included and responded appropriately. No evidence of cytotoxicity was observed. No information was provided regarding test substance precipitation. No increase in revertants was observed at any concentration with or without metabolic activation.

In an in vivo micronucleus assay, ICR mice (6 males/group) were exposed to P-15-0487 (purity: 90%) in DPPC solution at concentrations of 12.5, 25 or 50 mg/kg-bw. No signs of systemic toxicity or cytotoxicity were observed. Negative (vehicle) and positive controls were included and responded appropriately. The test item did not induce micronuclei in male or female mice exposed to P-15-0487 under the conditions of this study.

Chinese hamster ovary (CHO-k1) cells were exposed to P-15-0487 (purity: > 90%) in DPPC solution at concentrations ranging from 0.78 to 3.13 µg/mL, with and without metabolic activation. Negative (vehicle) and positive controls were included and responded appropriately. In a preliminary range-finding test, cytotoxicity was observed at concentrations ≥ 3.13 µg/mL in the presence and absence of metabolic activation, and test substance precipitation was observed at concentrations ≥ 6.25 µg/mL. No information regarding cytotoxicity or test substance precipitation was reported in the main study. No increase in the number of aberrant metaphases was observed at any concentration with or without metabolic activation.

Study 1: Acute oral toxicity study in rats

Title: Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method)

Ahn, K-S. 2014. Acute Oral Toxicity Study of MWCNT in Sprague-Dawley Rats (Acute Toxic Class Method). Performing Laboratory: [redacted] ponsor: [redacted].

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
P-15-0487 in DPPC solution (5.5 mM D-(+)-glucose, 0.6 mg/mL bovine serum albumin, and 0.01 mg/kg [redacted] in Dulbecco's phosphate buffered saline) was administered via gavage at 300 mg/kg-bw in a volume of 30 mL/kg to two groups of three female Sprague-Dawley (SD) rats. The test substance (volume of 10 mL/kg) was administered three times on a single day with 2-3 hour intervals between each administration (total dosing volume of 30 mL/kg). The first group of rats was dosed and observed for 14 days. As no mortality or clinical signs of toxicity were observed within 6 days in the first set of rats, the second group of three female rats was dosed and observed for 14 days. After the 14-day observation period, the animals were sacrificed, and a gross pathological examination was conducted. Dose selection was determined in accordance with OECD TG 423 and the dose level of the first step (300 mg/kg-bw) was selected due to an absence of available toxicity information on the test substance. Justification for the choice of vehicle was based on Kim et al., 2011 [1] which showed the test substance was equally dispersed up to 1% in [redacted] solution. No statistical analyses were performed. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2012-23 (2012) and OECD TG 423 (2001) and conformed to GLP standards. The study author noted that homogeneity and stability tests were not performed because the test solutions were prepared on the morning of administration. Administration of the test substance to two additional groups of rats at 2000 mg/kg-bw (the next step according to the dosing protocol in OECD TG 423) was not conducted due to the low solubility of the test substance in the vehicle (dispersion up to [redacted] solution). However, these deviations were not considered to have impacted the study results or conclusions.

Results and Discussion:
The acute oral LD50 was > 300 mg/kg-bw in female rats. No mortality or clinical signs of toxicity were noted. Body weight gain was normal in all animals throughout the study period. Necropsy revealed no macroscopic abnormalities.

[1] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

Conclusions:
Author's conclusions: Under the conditions of this study, the acute oral LD50 for female rats was > 300 mg/kg-bw.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 2: Acute dermal toxicity study in rats

Title: Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats

Ahn, K-S. 2014. Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats. Performing Laboratory: [redacted] Sponsor: [redacted]

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Sprague-Dawley (SD) rats (5/sex) were dermally exposed to a single dose of P-15-0487 at a dose level of 2000 mg/kg-bw and observed for 14 days. The PMN substance was applied to a 5x5 cm piece of gauze and moistened with [redacted] solution (5.5 mM D-(+)-glucose, 0.6 mg/mL Bovine serum albumin, and 0.01 mg/kg [redacted] in Dulbecco's phosphate buffered saline). The gauze pad was placed on the clipped, intact skin (~20% of total body surface area) of each rat and held in place for 24 hours with non-irritating tape and dressing bandages. An additional group of rats (5/sex) was exposed to [redacted] solution only. Following the exposure period, the dressings were removed and the test site was washed with sterile distilled water. Test animals were sacrificed at the end of the study, and a gross pathological examination was conducted. Dose selection was determined in accordance with OECD TG 402. Justification for the choice of vehicle was based on Kim et al., 2011 [2] which showed the test substance was equally dispersed up to 1% in [redacted] solution. Body weights were analyzed using the Independent Samples t-Test. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-1 (2013) and OECD TG 402 (1987) and conformed to GLP standards. The study author indicated that a stability test was not performed because the vehicle solution was only used to wet the PMN substance prior to application. However, the lack of stability testing was not considered to have impacted the study results or conclusions.

Results and Discussion:
The acute dermal LD50 was > 2000 mg/kg-bw in male and female rats. No mortalities occurred. No signs of toxicity or skin irritation were noted during the study. Average body weights were slightly decreased (1-4%) in all animals of both the vehicle control and treatment groups one day following administration, as compared to body weights prior to administration. There were no statistically significant differences in body weight between the vehicle control and treatment groups. All animals gained body weight throughout the remainder of the observation period. Necropsy revealed no macroscopic abnormalities.

[2] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

Conclusions:
Author's conclusions: Under the conditions of this study, the acute dermal LD50 was estimated to be > 2000 mg/kg-bw in male and female rats.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 3: Acute inhalation toxicity study in rats

Title: Acute Inhalation Toxicity Study of MWCNT in Fischer 344 Rats

Choi, B-G. 2014a. Acute Inhalation Toxicity Study of MWCNT in Fischer 344 Rats. Performing Laboratory: [redacted] Sponsor: [redacted]

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fischer 344 rats (5/sex/group) were exposed whole-body to P-15-0487 for 6 hours at target concentrations of 0 (filtered fresh air), 0.0002, 0.0005, and 0.001 mg/L (measured concentrations of 0, 0.00017, 0.00052, and 0.00083 mg/L, respectively) and observed for 14 days. The highest exposure concentration was the maximal mass concentration capacity for the carbon nanotube generating system; low- and mid-concentrations were determined by the standard high-concentration dilution process. The geometric mean cumulative median length (±SD) of the PMN substance was 233.97±1.57 nm. Body weights and results of lung function tests were analyzed by one way analysis of variance and Dunnett's test. Statistical analyses were conducted with a minimum significance level of 5%. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-02 (2013) and OECD TG 403 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The 6-hour LC50 was > 0.00083 mg/L. No treatment-related mortality or signs of toxicity were observed during the study. There were no significant effects on body weights. No treatment-related effects were observed at necropsy.

Conclusions:
Author's conclusions: The 6-hour LC50 was > 0.00083 mg/L in male and female rats.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 4: 28-day inhalation toxicity study in rats

Title: Subacute Inhalation Toxicity Study of MWCNT in Fischer 344

Choi, B-G. 2014b. Subacute Inhalation Toxicity Study of MWCNT in Fischer 344. Performing Laboratory: [redacted] Sponsor: [redacted]

Test Substance Identity: P-15-0487; black powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fisher 344 rats (10/sex/group) were exposed nose-only to P-15-0487 for 6 hours/day, 5 days/week, for 28 consecutive days at target concentrations of 0 (filtered fresh air), 0.0002, 0.0005, or 0.001 mg/L (analytically measured concentrations: 0, 0.00017, 0.00051, and 0.00097 mg/L, respectively). Target exposure concentrations were selected based on the results of an acute inhalation toxicity study in rats (Choi, 2014a, reviewed herein) in which no mortality or toxic signs were observed up to 0.00083 mg/L, the highest concentration tested. The highest exposure concentration was the maximal mass concentration capacity for the carbon nanotube generating system. The geometric mean cumulative median length (±SD) of the PMN substance was 395.33±1.51 nm. Continuous data were analyzed using the standard one-way analysis of variance and Duncan's or Dunnett's test. Non-continuous data were analyzed by Chi-squared analysis. Statistical analyses were conducted with a significance level of $p < 0.05$. The study, which was conducted in 2013, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-2 (2013) and OECD TG 412 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The NOAEC for male and female rats (determined by the reviewer) is 0.00097 mg/L, based on no adverse exposure-related effects observed at the highest concentration tested. A LOAEC could not be determined.

No mortalities occurred. There were no PMN substance-related effects on clinical observations, body weight, body weight change, food consumption, hematology, urinalysis, organ weights, gross findings, or histopathological findings. Food consumption of high-concentration males was significantly decreased by 15%, compared with controls, during week 1. Food consumption of high-concentration females was significantly increased by 11% during week 4. The changes in food consumption were not considered to be treatment-related as body weight and body weight change were not affected. Significantly increased (13%) magnesium levels were observed in males' blood of the high-concentration group. There was a concentration-related trend for increased magnesium levels as an increase of 8% was observed in males of the mid-concentration group. Potassium levels were also significantly increased (7%) in males of the high-

concentration group. Plasma glucose levels were decreased in a concentration-dependent manner in male rats from the mid- and high-concentration groups (11% and 13%) when compared with the control group. Plasma glucose levels for males of the high-concentration group were statistically significantly different from control levels. Albumin/globulin ratio was significantly decreased by 5% in male rats from the low- and high-concentration groups when compared to the control. The study author stated that the clinical chemistry changes were not treatment-related as the values either fell within the normal physiological range and/or the changes were not statistically concentration-dependent. No data demonstrating the normal physiological range for clinical chemistry were included in the study report. However, the biological relevance of the clinical chemistry changes is unknown as there were no correlating changes in organ weights or histopathology. Histopathological changes were observed at a similar incidence in the control and high-concentration animals and were not considered to be PMN substance-related.

Conclusions:
Author's conclusions: Exposure to the test substance for 28 days did not have any significant health effects on the rats in this study.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion. The study author did not determine LOAEC and NOAEC values in the study. The reviewer considers the NOAEC for male and female rats to be > 0.00097 mg/L, as no adverse effects were observed at the highest concentration tested. The LOAEC could not be established. It is noted that the highest concentration tested did not result in toxic effects, but the highest concentration was the maximal mass concentration capacity for the carbon nanotube generating system.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 5: 90-day inhalation toxicity study in rats

Title: Subchronic Inhalation Toxicity Study of MWCNT in Fischer 344

Choi, B-G. 2014c. Subchronic Inhalation Toxicity Study of MWCNT in Fischer 344. Performing Laboratory: [redacted] Sponsor: [redacted]

Test Substance Identity: P-15-0487; black powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
Fisher 344 rats (10/sex/group) were exposed nose-only to P-15-0487 for 6 hours/day, 5 days/week, for 13 weeks at target concentrations of 0 (filtered fresh air), 0.0002, 0.0005, or 0.001 mg/L (analytically measured concentrations: 0, 0.00017, 0.00051, and 0.00101 mg/L, respectively). Additional male rats (5/group) were included at all concentrations for the assessment of recovery after a 13-week non-exposure period. Target exposure concentrations were selected based on the results of an acute inhalation toxicity study in rats and a 28-day repeated-dose inhalation study in rats (Choi, 2014a, b; reviewed herein) in which no mortality or toxic signs were observed at the highest concentrations tested, 0.00083 mg/L and 0.00097 mg/L, respectively. The highest exposure concentration for this study was the maximal mass concentration capacity for the carbon nanotube generating system. The geometric mean cumulative median length (±SD) of the PMN substance was 566.54±1.88 nm. Continuous data were analyzed using the standard one-way analysis of variance and Duncan's or Dunnett's test. Non-continuous data were analyzed by Chi-squared analysis. Statistical analyses were conducted with a significance level of $p < 0.05$. The study, which was conducted in 2014, followed the Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER), Notice No. 2013-2 (2013) and OECD TG 413 (2009) and conformed to GLP standards. Deviations from the study protocol were not specified.

Results and Discussion:
The NOAEC for male and female rats is > 0.00101 mg/L, based on no adverse treatment-related effects observed at the highest concentration tested. A LOAEC could not be determined.

One animal in the mid-concentration group died on study day 81 after exhibiting restlessness, convulsions, and stupor on study day 80. The animal had red exudate in the abdominal cavity; inflammation, flattening of the uroepithelium, lumen dilatation, and red urine in urinary bladder; pulmonary and hepatic congestion; focal mineralization of the renal tubule; and prostate edema, hemorrhage, and inflammation. The death was not considered to be related to exposure.

No other mortalities or clinical signs were observed in the main study or during the recovery period. In the main study, there were no PMN substance-related effects on body

weight, food consumption, ophthalmoscopy, hematology and blood coagulating parameters, female urinalysis parameters, female absolute organ weights, relative organ weights, gross necropsy findings, bronchoalveolar lavage test, or microscopic findings. During the recovery period, there were no differences in body weight, food consumption, ophthalmoscopy, hematology parameters, urinalysis parameters, absolute and relative organ weights, gross necropsy findings, bronchoalveolar lavage test, or microscopic findings among the groups.

In female rats of the main study, significantly increased ($p<0.05$) sodium levels (2%) were observed at all concentrations and significantly increased potassium (6%) levels were observed at the mid- and high-concentrations, compared with controls. During the recovery period, prothrombin time was significantly ($p<0.05$) increased by 10 and 9%, in males of the mid- and high-concentration groups, respectively. In males of the low- and high-concentration recovery groups, cholesterol levels were significantly increased by 8 and 7%, respectively, and magnesium levels were significantly decreased by 16 and 13%, respectively. In the main study, there was a statistically significant increase ($p<0.05$) in trace urine ketone bodies in males of the mid- and high-concentration groups; incidence of 0/5, 1/5, 3/5, and 5/5 at control, low-, mid-, and high-concentration, respectively. The incidence of urine ketone bodies (grade 1+) was 5/5, 4/5, 1/5, and 0/5 at control, low-, mid-, and high-concentration, respectively, showing a concentration-related decreasing trend with statistical significance in the mid- and high-concentration groups. A concentration-related trend for increasing urine pH values in PMN substance-exposed males was also observed. The findings were of uncertain toxicological relevance due to lack of gross and histological findings in the kidney. A statistically significant increase (18%) in absolute left lung weight was observed in males of the high-concentration group. The study author stated that the significant blood coagulation, clinical chemistry, and organ weight changes were not treatment-related as the values either fell within the normal physiological range and/or the changes were not concentration-dependent. No data demonstrating the normal physiological range were included in the study report. However, the biological relevance of the blood coagulation, clinical chemistry, and urine ketone body and pH changes is unknown as there were no correlating changes in organ weights or histopathology. Histopathological changes were observed in one or two animals or at a similar incidence in the control and exposed males and were not considered to be PMN substance-related.

Conclusions:
Author's conclusions: The NOAEC for male and female rats is > 0.00101 mg/L, and a target organ was not identified.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion that the NOAEC for male and female rats is > 0.00101 mg/L, based on no adverse treatment-related effects observed at the highest concentration tested; the LOAEC could not be determined. It is noted that the highest concentration tested did not result in toxic effects, but the highest concentration was the maximal mass concentration capacity for the carbon nanotube generating system. It is also noted that no females were included in the recovery group, and justification was not provided.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 6: In vitro genetic mutation study in bacteria

Title: Bacterial Reverse Mutation Test of MWCNT

Kim, J.S. 2014. Bacterial Reverse Mutation Test of MWCNT. Performing Laboratory: [redacted] Sponsor: [redacted]

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
A GLP-compliant bacterial reverse mutation assay with Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA was conducted using the preincubation method. This study complied with OECD TG 471 (1997) and National Institute of Environment Research (NIER) Notice No. 2012-23 (revised August 22, 2012). The strains were supplied by Molecular Toxicology Incorporated. In the preliminary range-finding test and main study, TA98, TA100, TA1535, TA1537, and WP2uvrA were exposed to the PMN substance in the vehicle [redacted]) at 0, 31, 63, 125, 250, or 500 µg/plate, with and without metabolic activation. The rationale for the selection of the concentrations was not reported. Both tests were conducted in triplicate. The positive controls in the absence of metabolic activation were the following: [redacted](TA98, TA100, and WP2uvrA), sodium azide (TA1535), and 9-aminoacridine hydrochloride hydrate (TA1537). In the presence of metabolic activation, [redacted] was the positive control for all strains. All positive controls were dissolved in dimethyl sulfoxide. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence and absence of metabolic activation were calculated. Justification for the choice of vehicle was based on Kim et al., 2011 [3] which showed the test substance was equally dispersed up to 1% in [redacted] solution. No deviations from the study protocol were noted.

Results and Discussion:
No increase in revertants was observed with or without metabolic activation in the main study. No evidence of cytotoxicity was observed in the range-finding test and main study. No information was provided regarding test substance precipitation. The vehicle and positive control data were within or close to the ranges established by the laboratory's historical data.

[3] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

Conclusions:
Author's conclusions: Under the conditions of this study, the test substance did not induce gene mutations.

Reviewer's conclusions: The reviewer agrees with the study author's conclusions that the test substance is negative for mutagenicity under the conditions of the study.

The reviewer notes the following study deficiencies or deviations from OECD TG 471: the test item was not tested at the recommended maximum concentration of 5 mg/plate; the number of cells per bacterial culture was not provided; 2-aminoanthracene was used as the sole indicator of the efficacy of the S9-mix; and no historical control data were provided although the authors did provide ranges of the acceptable number of revertants for vehicle and positive controls.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 7: In vivo bone marrow erythrocyte micronucleus test in mice

Title: Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR Mice

Kim, J.S. 2014. Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice. Performing Laboratory: [redacted] Laboratory Study No. [redacted]. Sponsor: [redacted]

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
A GLP-compliant in vivo bone marrow erythrocyte micronucleus test was conducted using ICR mice. This study complied with OECD TG 474 (1997) and National Institute of Environment Research (NIER) Notice No. 2012-23 (revised August 22, 2013). In the main test, ICR (CrljOri: CD1) mice (6 males/group) were administered the PMN substance in the vehicle [redacted] at 0, 12.5, 25, or 50 mg/kg-bw. The maximum dose was chosen to be 50 mg/kg-bw because no mortalities were observed at this dose in a preliminary range-finding test. A positive control group comprised of 6 male mice received 2.0 mg/kg-bw mytomycin-C. Bone marrow was harvested at 24 hours after PMN substance administration, and at least 2000 erythrocytes were evaluated per animal by microscopic examination for the number of micronucleated polychromatic erythrocytes (MNPCE). At least 200 erythrocytes were evaluated per animal to determine the numbers of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). The ANOVA test was used to compare the micronuclei frequency and PCE/(PCE+NCE) ratios of the negative control and treated groups. In the event that statistical significance was established by the ANOVA test, linear logistic regression was used to test for dose-response. Additionally, the ANOVA test was used to evaluate the body weights of test animals at necropsy, and Dunnett T3 or Duncan's multiple range test was used if statistical significance was established. Justification for the choice of vehicle was based on Kim et al., 2011 [4] which showed the test substance was equally dispersed up to 1% in [redacted] solution. No deviations from the study protocol were noted.

Results and Discussion:
The PMN substance did not induce micronuclei in bone marrow erythrocytes in male mice. Administration of the PMN substance did not result in a decrease in the PCE/(PCE+NCE) ratio, indicating that cytotoxicity was not observed. No clinical signs or statistically significant changes in body weight were noted in the test groups compared to the negative control group. The positive control induced a statistically significant ($p <$

[4] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

0.05) increase in the frequency of MNPCEs and a statistically significant ($p < 0.05$) decrease in the PCE/(PCE+NCE) ratio.

Conclusions:
Author's conclusions: The test item did not induce micronuclei in the bone marrow erythrocytes of the ICR mouse.

Reviewer's conclusions: Based on the reported results, micronuclei were not induced in mice bone marrow. Due to numerous deficiencies in reporting, as well as deviations from OECD TG 474, the reviewer considers the results of this study to be inconclusive.

The reviewer notes the following study deficiencies or deviations from OECD TG 474: the highest dose tested did not cause toxicity in the main study and a sufficient justification was not provided for the selection of doses; no justification was provided for only testing male animals; samples of bone marrow were collected only once instead of twice within 48 hours; the same treatment regimen may not have been used in the preliminary study (oral administration) and main study (oral or i.p. injection- unclear in report); rationale for route of administration was not provided; inconsistent reporting of doses used in the preliminary test [500, 1000, and 2000 mg/kg-bw (p. 10 of study report) vs. 12.5, 25, and 50 mg/kg-bw (p. 6 of study report)]; inconsistent reporting of methods [e.g., test substance dispersed in distilled water (p. 10 of study report) vs dispersed in [redacted] solution (p. 4 of study report); negative control group received corn oil (p. 6 of study report) vs. [redacted] solution (Tables 1-2, pp. 12-13)]; and no methods were used to verify that the test substance reached the target tissue.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.

Study 8: In vitro mammalian chromosome aberration

Title: In vitro Mammalian Chromosome Aberration Test of MWCNT Using Cultured Chinese Hamster Ovary (CHO-k1) Cells

Kim, J.S.. 2014. In vitro Mammalian Chromosome Aberration Test of MWCNT Using Cultured Chinese Hamster Ovary (CHO-k1) Cells. Performing Laboratory: [redacted] Laboratory Study No. [redacted]. Sponsor: [redacted]

Test Substance Identity: P-15-0487; powder; purity: > 90%; Trade name: K-Nanos-100P; referred to as multi-wall carbon nanotube (MWCNT) in the study report

Methods:
A GLP-compliant in vitro mammalian chromosome aberration test was conducted with Chinese hamster ovary (CHO-k1) cells. This study complied with OECD TG 473 (1997) and National Institute of Environment Research (NIER) Notice No. 2012-23 (revised August 22, 2012). The CHO-k1 cells were obtained from Korean Cell Line Bank. In the preliminary range-finding test, CHO-k1 cells were exposed to the PMN substance in the vehicl [redacted] at 0 (vehicle control), 1.56, 3.13, 6.25, 12.5, 25, 50, 100 or 200 µg/mL in the absence of metabolic activation for 6 or 24 hours, or in the presence of metabolic activation for 6 hours. Based on the results of the range-finding experiment, in the main test, CHO-k1 cells were exposed to the PMN substance in the vehicle at 0 (vehicle control), 0.78, 1.56 or 3.13 µg/mL in the absence of metabolic activation for 6 or 24 hours, or in the presence of metabolic activation for 6 hours. Duplicate cultures were tested at each concentration for each experimental condition. All cells were harvested at 24 hours. The positive control in the absence of metabolic activation was mitomycin C. The positive control in the presence of metabolic activation was cyclophosphamide. The percentage of aberrant metaphases, excluding gaps, and frequency of cells with polyploidy or endoreduplication at each concentration were calculated for each experimental condition. Statistical significance, as compared to the vehicle control, was determined using a Chi-squared test. A linear regression test was performed for dose-response. Justification for the choice of vehicle was based on Kim et al., 2011 [5] which showed the test substance was equally dispersed up to 1% in [redacted] solution. No deviations from the study protocol were noted.

Results and Discussion:
In the range-finding test, cytotoxicity (>50% decrease in relative cell count, defined by the reviewer) was observed at concentrations ≥ 3.13 µg/mL and precipitation was observed at ≥ 6.25 µg/mL, with and without metabolic activation. In the main test, the

[5] Kim, J.S., Song, K.S., Lee, J.H., Yu, I.J. 2011. Evaluation of biocompatible dispersants for carbon nanotube toxicity tests. Arch Toxicol, 85: 1499-1508.

test substance did not induce a statistically significant increase in the frequency of chromosome aberrations in CHO-k1 cells exposed for 6 or 24 hours without metabolic activation or 6 hours with metabolic activation. Additionally, the test substance did not induce a statistically significant increase in the frequency of polyploidy or endoreduplication under any treatment condition. No information was provided regarding observations of cytotoxicity or precipitation in the main test. In the negative (vehicle) control group, the frequency of chromosome aberrations ranged from 0-0.5. The positive controls induced statistically significant ($p < 0.05$) increases in the frequency of chromosome aberrations under all test conditions.

Conclusions:
Author's conclusions: Under the conditions of this study, the test substance did not induce chromosome aberrations in Chinese hamster ovary (CHO-k1) cells.

Reviewer's conclusions: The reviewer agrees with the study author's conclusion that the test substance is negative for clastogenicity under the conditions of the study.

The following study deficiencies or deviations from OECD TG 473 were noted: cytotoxicity criteria were not clearly defined in the report, although the highest analyzable concentration, 3.13 µg/mL, caused a >50% decrease in relative cell count; no historical vehicle or positive control data were provided; and the study report did not specify the incubation temperature during testing.

EPA conclusions: Based on our evaluation of the test results, RAD supports the conclusions made by the reviewer.